Standard operating procedures (SOPs) for new POPs

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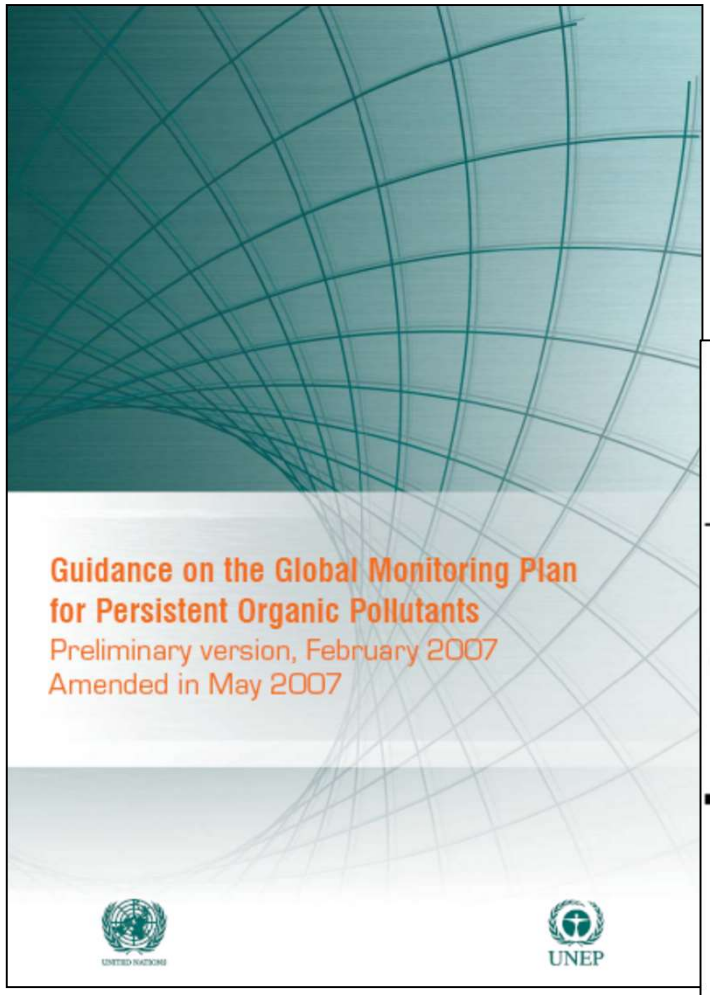
New POP compounds to be analysed

POP	Air	Human milk/blood	Water
Chlordecone	Chlordecone		
Endosulfan	α-, β-endosulfan; and endosulfan sulfate		
HBCD	α-HBCD, β-HBCD, γ-HBCD		
Hexachloroyclohexanes	α-HCH, β-HCH, γ-HCH		
Hexabromobiphenyl	PBB-153		
Pentachlorobenzene	PeCBz		
c-penta BDE, c-octa BDE	PBDE 47, 99, 153, 154, 175/183 (co-eluting		
	Optional: PBDE 17, 28, 100	Optional: PBDE 100	
PFOS	PFOS (linear and sum of PFOS)		
	NMeFOSA, NEtFOSA, NMeFOSE, NEtFOSE		

HF, New POPs Tools, Hanoi, Jan 2016

Guidance for Global Monitoring Plan



Orientation and benchmark for POPs www.pops.int

UNITED NATIONS

UNEP

SC

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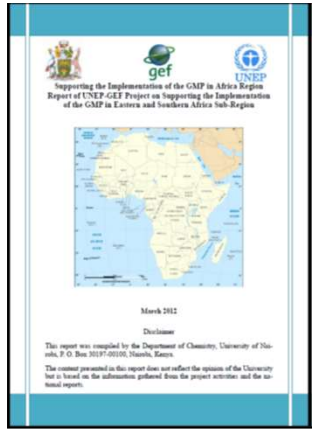
Stockholm Convention on Persistent Organic Pollutants

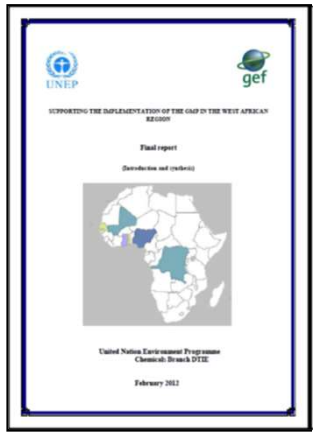
Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants
Seventh meeting
Geneva, 4–15 May 2015
Item 5 (i) of the provisional agenda*
Matters related to the implementation of the Convention: effectiveness evaluation

Guidance on the global monitoring plan for persistent organic pollutants

POPs Analysis and Monitoring

	Regional and national reports	Training reports
Pacific Islands Region	- GMP Regional Report of Pacific Islands Region - GMP National Report of Kiribati - GMP National Report of Marshall Islands - GMP National Report of Niue - GMP National Report of Palau - GMP National Report of Solomon Islands - GMP National Report of Samoa	- Fiji Training Report
GRULAC Region	- GMP Regional Report of GRULAC Region (en, sp) - GMP National Report of Antigua and Barbuda - GMP National Report of Brazil - GMP National Report of Chile - GMP National Report of Ecuador - GMP National Report of Jamaica - GMP National Report of Mexico (sp) - GMP National Report of Peru (sp)	- Regional Report for GRULAC - Reports of Antigua and Barbuda (en, sp); Brazil (sp); Chile (sp); Ecuador (sp); Jamaica (en, sp); Mexico (sp); Peru (sp); Uruguay (sp)
East and South Africa	- GMP Regional Report of E+S Africa - GMP National Report of Egypt - GMP National Report of Ethiopia - GMP National Report of Kenya - GMP National Report of Mauritius - GMP National Report of Uganda - GMP National Report of Zambia	- Egypt Training Report - Kenya Training Report - Mauritius Training Report - Zambia Training Report
West Africa	- GMP Regional Report of West Africa (en, fr) - GMP National Report of DR Congo (fr) - GMP National Report of Ghana - GMP National Report of Mali (fr) - GMP National Report of Nigeria - GMP National Report of Senegal (fr) - GMP National Report of Togo (fr)	- Ghana Training Report - Mali Training Report - Senegal Training Report
Cross-cuttings	- IVM Mirror samples Final Report (Africa, Pacific, Barbados) - MTM Report. Analysis of dl POPs in PUF samples (Africa and Pacific Islands) - MTM Report dl-POPs in National Samples - UNEP Report: Passive air sampling (PAS)	
Interlaboratory Assessments	- Biennial Global Interlaboratory Assessment on POPs – Round 1	



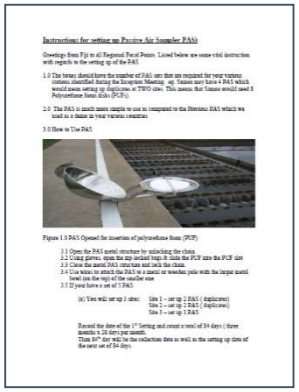


POPs Analysis and Monitoring

SOPs and supporting materials

Pacific Islands Region

- SOP Regional Guidance for Mothers Collecting Milk Samples
- USP-IAS Instructions for PAS



GRULAC Region

- Guide for PAS (en, sp)
- SOP Cleaning of glassware (en, sp)
- SOP Collection of mothers' milk (en, sp)
- SOP Indicator PCB in air (en, sp)
- SOP Indicator PCB in fish (en, sp)
- SOP Indicator PCB in mothers' milk (en, sp)
- SOP OCP en aire (en, sp)
- SOP OCP en leche materna (en, sp)
- SOP OCP en pescado (en, sp)
- SOP OCP en sedimentos (en, sp)
- SOP PCDD PCDF dl-PCB en aire (en, sp)
- SOP PCDD PCDF dl-PCB en leche materna (en, sp)
- SOP PCDD PCDF dl-PCB en pescado (en, sp)
- SOP PCDD PCDF dl-PCB en sedimentos (en, sp)

East and South Africa

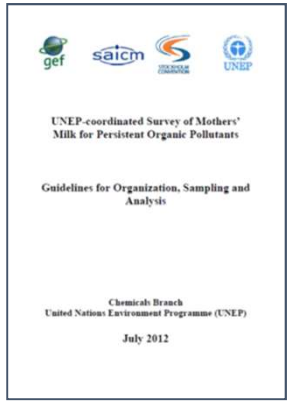
- SOP Kenya: Mothers' Milk
- SOP Recetox PAS

West Africa

- SOP in passive air sampling (PAS)

Cross-cuttings

- Guidance for organisation, sampling and analysis of human milk



http://www.unep.org/chemicalsandwaste/POPsandScience/AnalysisandMonitoring/GlobalMonitoringPlan/GMPImplementation2009-2012/tabid/1059888/Default.aspx

Laboratory instru-mentation level	Equipment	Infrastructure needs	Chemicals
5	Sample extraction and clean-up systems (manually or automated), LC-MS/MS)	Nitrogen/air condition-ing/consistent power/high operational costs/personnel specifically trained to operate and troubleshoot complicated instrumentation	PFOS and other anionic PFCs , PFOSA
3	Basic sample extraction and clean-up equipment, capillary GC-ECD	Nitrogen/air conditioning/power/ personnel specifically trained to operate and troubleshoot equip-ment problems	PBB, most PCB and all OCPs except toxaphene
2a	Sample extraction and clean-up equipment, capillary GC-LRMS – electron ionization mode	Helium/air conditioning/ consistent power/ personnel specifically trained to operate and trouble-shoot equipment problems	PBB, most PCB and all OCPs; Also perfluoro-sulfamido alcohols in positive chemical ionization mode
2b	Sample extraction and clean-up equipment, capillary GC-LRMS – negative chemical ionization mode	Methane or other moderating gas/air conditioning/ consistent power/ personnel specifically trained to operate and trouble-shoot equipment problems	PBDE and PBB, as well as toxaphene and other highly chlorinated (≥4 Cl) OCPs
1	Sample extraction and clean-up equipment, capillary GC-HRMS	Helium/air conditioning/ consistent power/high opera-tional costs /personnel spe-cifically trained to operate and troubleshoot complicated instrumentation	PCDD/PCDF, all PCB, all OCPs, PBB, all PBDE

GC-ECD – gas chromatography/electron capture detection
GC-LRMS – gas chromatography/low resolution mass spectrometry
GC-HRMS – gas chromatography/high resolution mass spectrometry
LC-MS/MS – high performance liquid chromatography/tandem mass spectrometry
PY – Person-year

Instrumentation – Tier

GMP guideline

Standard operational procedures for new POPs – example of PFAS

Method development

- In order to generate high quality and comparable results, the protocols and methods for sampling and analysis of all POPs in relevant types of samples have to be harmonized;
- In all regions and over time, the same basic approaches and quality criteria for acceptance of data and assessment of results should be applied.
  - Standard operating procedures (SOPs) for groups of POPs
  - General guidelines for specific matrices (types of samples):

Note: The guides and SOPs should be taken as an orientation and be transferred into daily routines by each laboratory.

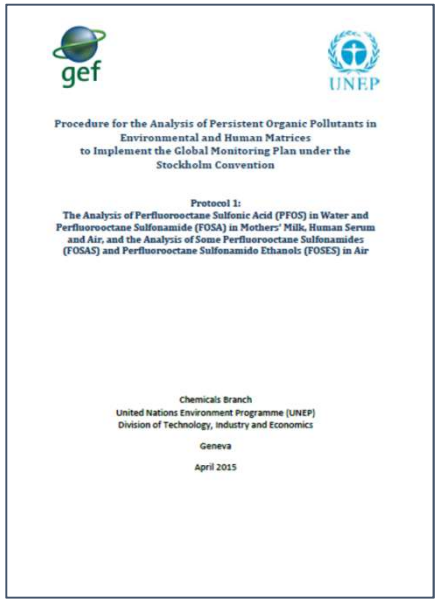
http://www.unep.org/chemicalsandwaste/POPsandScience/AnalysisandMonitoring/MethodDevelopment/tabid/1059865/Default.aspx

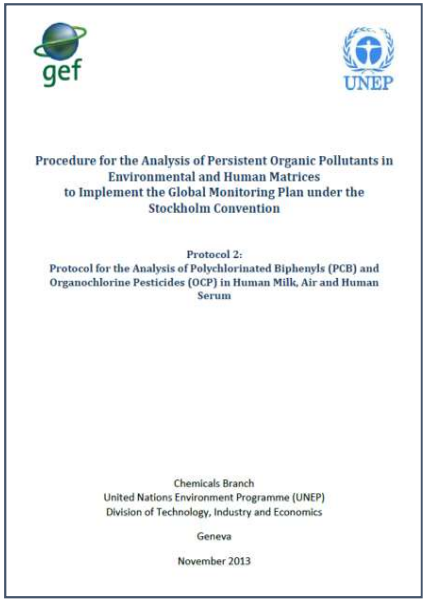
Choice of analytical method

- The SOPs prepared for UNEP describe general procedures for analysis;
- However, it is possible to change certain parameters and analytical conditions described in this protocol, while still obtaining the same results;
- In any case, the entire method should be optimized and validated to ensure the comparability of data.

SOPs for POPs

- Procedure for the Analysis of POPs – Protocol 1: Analysis of **PFOS in Water and FOSA** in Mothers' Milk Serum and Air, and the Analysis of some FOSAs and FOSEs in Air
- Procedure for the Analysis of POPs – Protocol 2: Analysis of **PCB and OCP** in Human Milk, Air and Human Serum
- Procedure for the Analysis of POPs – Protocol 3: Analysis of **PBDE** in Human Milk, Air and Human Serum





http://www.unep.org/chemicalsandwaste/POPsandScience/AnalysisandMonitoring/MethodDevelopment/tabid/1059865/Default.aspx

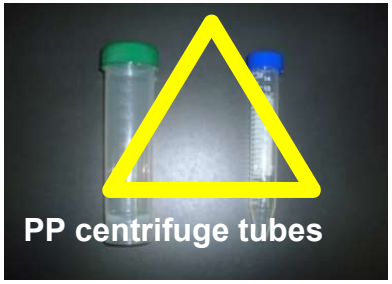
INTERNATIONAL STANDARD

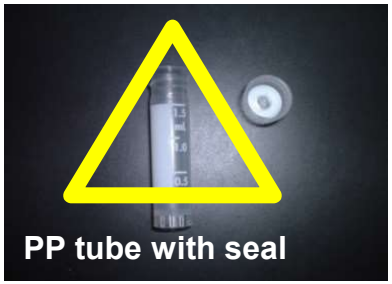
ISO 25101

First edition
2009-03-01

Water quality — Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) — Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry

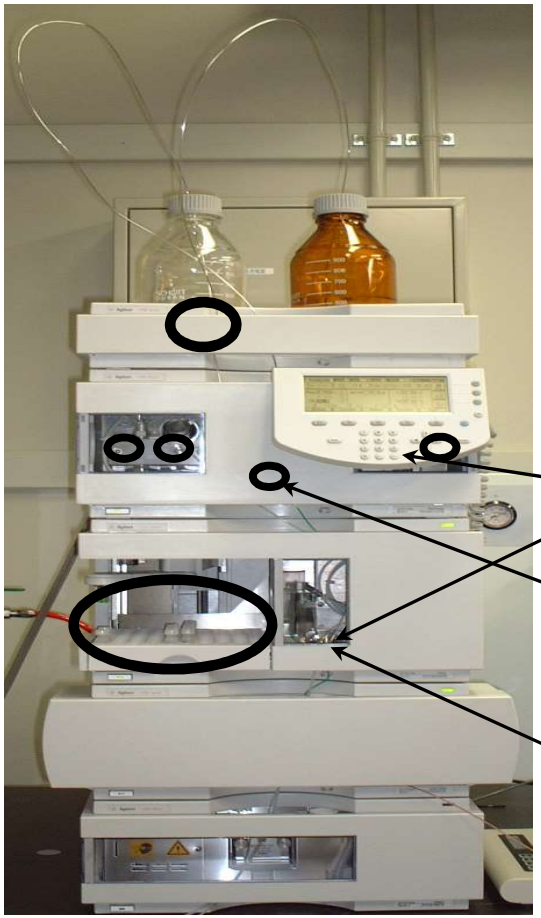
How to control background contamination in the laboratory?





How to control background contamination from the instrument?

How to control instrumental blank?



seal

solvent selection valve

rotor seal

Septum blank?

POLYETHYLENE

TEFLON

VITON

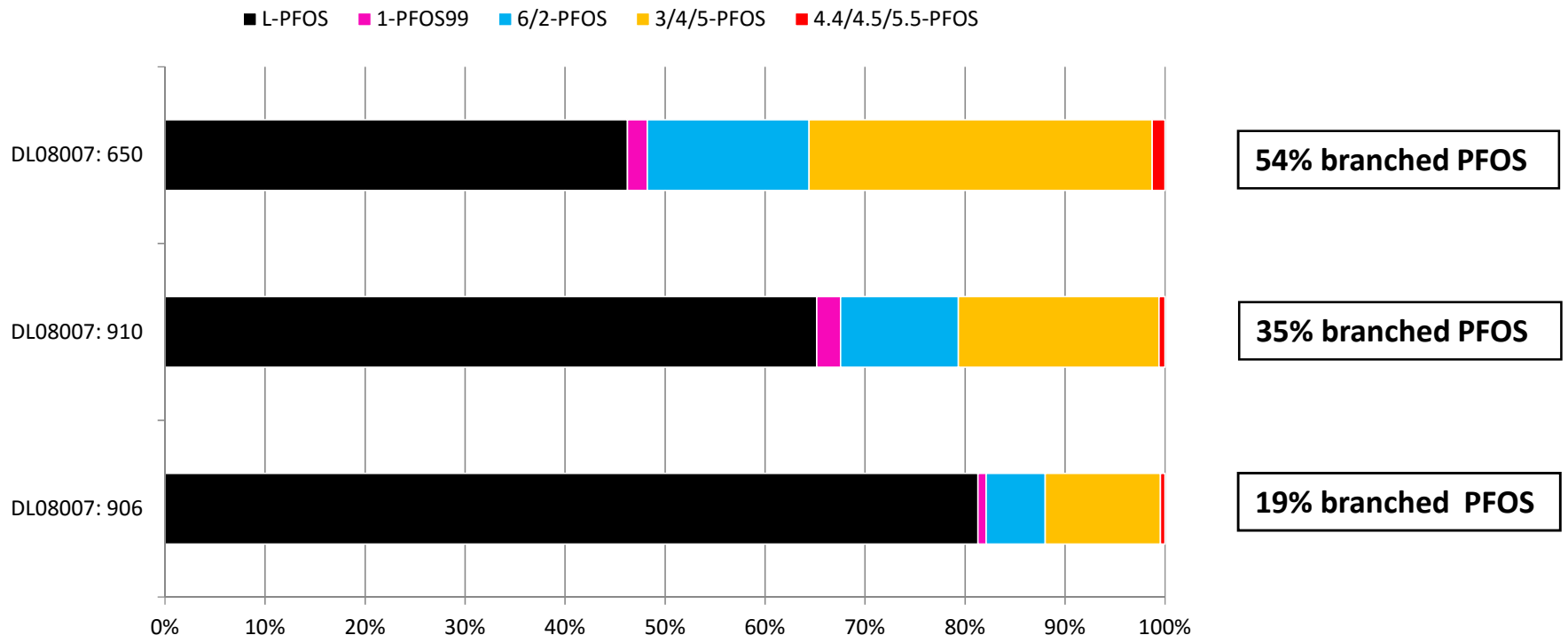
Structural isomers of PFOS identified in technical mixtures

Abbreviation	Formula	Name
L-PFOS	$CF_3CF_2CF_2CF_2CF_2CF_2CF_2CF_2SO_3^-$	*n*-perfluoro-octanesulfonate
1-PFOS	$CF_3CF_2CF_2CF_2CF_2CF_2CF(\mathbf{CF_3})SO_3^-$	perfluoro-1-methyl-heptanesulfonate
2-PFOS	$CF_3CF_2CF_2CF_2CF_2CF(\mathbf{CF_3})CF_2SO_3^-$	perfluoro-2-methyl-heptanesulfonate
3-PFOS	$CF_3CF_2CF_2CF_2CF(\mathbf{CF_3})CF_2CF_2SO_3^-$	perfluoro-3-methyl-heptanesulfonate
4-PFOS	$CF_3CF_2CF_2CF(\mathbf{CF_3})CF_2CF_2CF_2SO_3^-$	perfluoro-4-methyl-heptanesulfonate
5-PFOS	$CF_3CF_2CF(\mathbf{CF_3})CF_2CF_2CF_2CF_2SO_3^-$	perfluoro-5-methyl-heptanesulfonate
6-PFOS	$CF_3CF(\mathbf{CF_3})CF_2CF_2CF_2CF_2CF_2SO_3^-$	perfluoro-6-methyl-heptanesulfonate
4,4-PFOS	$CF_3CF(\mathbf{CF_3})_2CF_2CF_2CF_2CF_2SO_3^-$	perfluoro-4,4-dimethyl-hexanesulfonate
3,5-PFOS	$CF_3CF(\mathbf{CF_3})CF_2CF(\mathbf{CF_3})CF_2CF_2SO_3^-$	perfluoro-3,5-dimethyl-hexanesulfonate
4,5-PFOS	$CF_3CF(\mathbf{CF_3})CF(\mathbf{CF_3})CF_2CF_2CF_2SO_3^-$	perfluoro-4,5-dimethyl-hexanesulfonate
5,5-PFOS	$CF_3C(\mathbf{CF_3})_2CF_2CF_2CF_2CF_2SO_3^-$	perfluoro-5,5-dimethyl-hexanesulfonate

Technical mixtures typically contain between 71% and 83% L-PFOS (Vyas et al. 2007)

Differences in PFOS isomer profiles in human plasma

Why do we see differences?
-sources of exposure
-historical vs. recent exposure
-precursors
-differences internal elimination



Choice of analytical method

Table 2. A summary of selected analytical methods for the determination of fluorinated POPs in human serum/plasma

Analytes	*N* analytes	Study type	Year	Sample preparation			Instrumental analysis			
				Sample amount	Pre-treatment	Extraction and clean-up	Configuration	Injection volume	Separation	Ref.
PFAAs	13	Method development	2011	100 µL	0.1 M formic acid	On-line SPE: C18	HPLC-ESI-MS/MS	400 µl	Betasil C8	[41]
PFAAs	13	Inter-laboratory comparison	2010	0.15–1.2 g	Formic acid (50/50, v/v)	SPE: Oasis WAX	HPLC-ESI-MS/MS	5–20 µl	Betasil C8	[38]
PFAAs	10	Inter-laboratory comparison	2010	0.2 mL	0.1 mol/L formic acid	On-line SPE: C18	HPLC-TIS-MS/MS	n.i.	Betasil C8	[38]
PFAAs	13	Inter-laboratory comparison	2010	0.2 mL	PP with acetonitrile	n.i.	HPLC-TIS-MS/MS	n.i.	Betasil C18, Prism RP C12	[38]
PFAAs	8	Inter-laboratory	2010	1 mL	n.i.	LLE using ion pair,	HPLC-ESI-MS/MS	n.i.	ACE C18	[38]
PFAAs	9									[38]
PFAAs	11									[37]
PFAAs	19									[40]
PFAAs	25	Method development	2010	0.5 mL whole blood	Formic acid (50% in water) or acetonitrile	SPE: Oasis WAX, LLE using ion pair, and LLE using acetonitrile	LC-ESI-MS/MS	10 µl	Betasil C18 and JJ-50 2D	[36]
PFAAs	18	Method development	2005	100 µL	0.1 M formic acid	On-line SPE: C18	HPLC-TIS-MS/MS	400 µl	Betasil C8	[34]
PFAAs	12	Method development	2005	0.75 mL whole blood	Formic acid (50/50, v/v)	SPE: C18, filtration with membrane 0.2 µm filter	HPLC-ESI-MS and LC-MS/MS	10 µl	Discovery HS C18	[43]
PFAAs	13	Method development	2004	1 mL	0.1 M formic acid	Off-line SPE: Oasis HLB	HPLC-TIS-MS/MS	12 µl	Betasil C8	[39]

Note: no information (n.i.).

The choice of analytical method should be needs-oriented and developed to fit the purpose of the study!

Salihovic et al, TRAC 2013

Principle

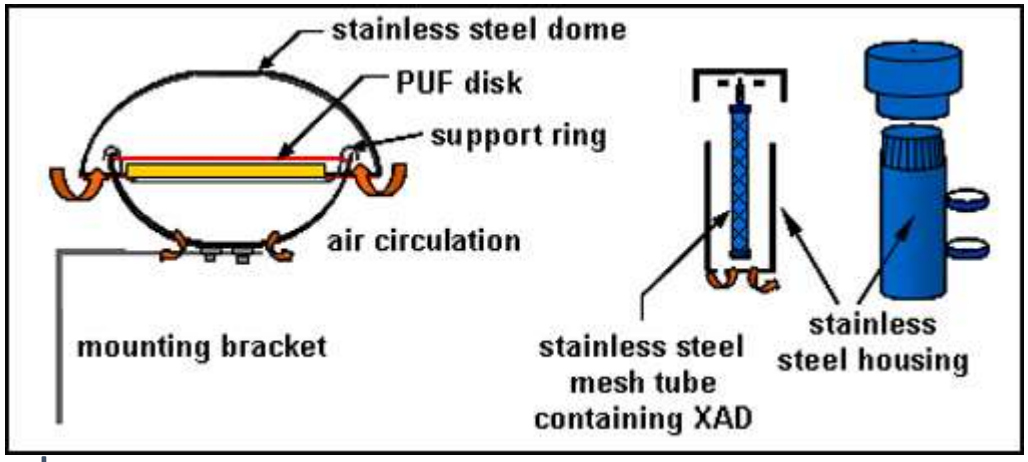
- Sample preparation of water, milk, and serum/plasma samples is similar but is different for air;
- Extraction involves Soxhlet extraction and SPE;
- Clean-up (ENV carb) is used depending on the complexity of the sample matrix;
- Method valididation using ILSs, SRMs, CRMs, and spiking experiments;
- Instrumental analysis performed using UPLC-ESI-MS/MS operated in negative ion mode.

Materials and reagents

- **Materials:**
- ✓ Glass Beaker (2 L)
- ✓ Polypropylene bottle (100 mL)
- ✓ Plastic pipettes
- ✓ Polypropylene tubes (15 mL)
- ✓ Micro tubes (1.5 mL)
- ✓ Crimpcap polypropylene vial (700 µL)
- ✓ Seal, silver aluminum 11 mm, PTFE/Rubber Liner
- ✓ Capper/Decapper
- ✓ Ultrasonic bath
- ✓ Vacuum dessiccator
- ✓ Passive sampler
- ✓ Balance (precision 0.01 g)
- ✓ Pipettes (50, 100 and 200 µL)
- ✓ Centrifuge
- ✓ Oven (37 °C)
- ✓ SPE device (rinse with methanol and water prior to use)
- ✓ pH meter
- ✓ Vacuum pump
- ✓ Water bath (50 °C)
- ✓ Whirlmixer
- ✓ LC-MS/MS (LC-QQQ). Electrospray source (ESI) with negative polarity
- ✓ FluoroSEP-RP Octyl column, 15 cm x 2.1 mm, 5 µm particle size, ES Industries (132211-FO)
- ✓ 2 x Symmetry columns C18, 20 mm x 3.9 mm, 5 µm particle size, Waters (WAT054225)
- ✓ Symmetry column C18, 50 mm x 2.1 mm, 5 µm particle size, Waters (18600206)

- **Reagents:**
- ✓ Polyurethane foam (PUF) disk, 14 cm x 1.35 cm, surface area 365 cm^2, mass 4.40 g, volume 207 cm3, Tisch Environmental, Cleves, OH
- ✓ Aceton, Ultraresi, J.T.Baker (9254)
- ✓ Petroleum ether, J.T.Baker
- ✓ Methanol, HPLC gradient grade, J.T.Baker (8402)
- ✓ Internal standard ($^{13}C_4$ PFOS + $^{18}O_2$ PFOSA + $^{2}H_3$ MeFOSA + $^{2}H_5$ EtFOSA + $^{2}H_7$ MeFOSE + $^{2}H_9$ EtFOSE) in methanol (100 ng/mL)
- ✓ Internal standard ($^{13}C_4$ PFOS + $^{18}O_2$ PFOSA) in methanol (100 ng/mL)
- ✓ 50% Formic acid in water
- ✓ SPE Cartridge, Oasis WAX 6cc, Waters 186002493
- ✓ Ammonia 25% p.a. purity
- ✓ 0.1% NH4OH in methanol; add 400 µL ammonia to100 mL methanol
- ✓ 2 % NH4OH in methanol; add 8 mL ammonia to 92 mL methanol
- ✓ HPLC water, HPLC analyzed, J.T. Baker (4218), or MilliQ purity
- ✓ Acetic acid 100 % pro analysis (p.a.) purity
- ✓ Ammonium acetate p.a. purity
- ✓ 25 mM Ammonium acetate; add 190 mg ammonium acetate to 100 mL water and adjust the pH to pH=4 with acetic acid
- ✓ Nitrogen gas. Purity 5.0
- ✓ Injection standard (1) (13C8 PFOS) in methanol/water (1:1, v/v) (150 ng/mL)
- ✓ Injection standard (2) (13C8 PFOS) in methanol/water (1:1, v/v) (50 ng/mL)
- ✓ Injection standard (3) (13C8 PFOS) in methanol/water (1:1, v/v) (25 ng/mL)
- ✓ Ammonium formate, (>99%), Fluka (09735)
- ✓ Ammonium formate buffer 5 mM: Dissolve 315 mg ammonium formate in 1 L HPLC water. Filter prior to use.
- ✓ PFAS calibration solutions (0.05, 0.25, 0.5, 5, 50, 100 ng/mL) in methanol/water (1:1, v/v)

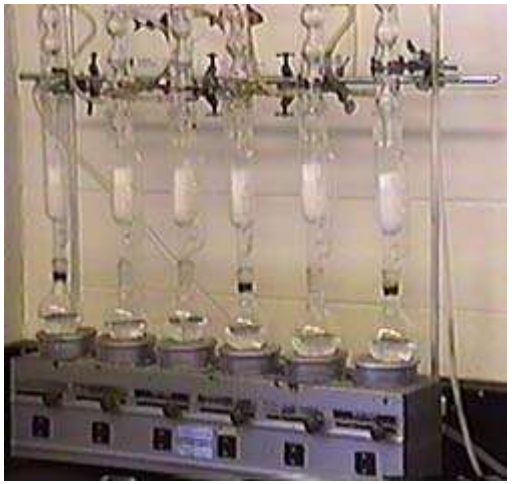
Air



- Polyurethane foam (PUF) disk
  - Preparation of the PUF
- Cleaning of a PUF:
  - If necessary, wash the PUF in water;
  - Perform a Soxhlet extraction on the PUF with acetone (24 h), followed by petroleum ether (24 h)
  - Dry the PUF in a desiccator (24 h).
- Air sampling
  - Place a PUF in a passive sampler for three months at sampling location.
- Sample preparation
  - Take the PUF out of the sampler;
  - Add 150 µL Internal standard (I.S.) to the PUF.
- Procedural blank
  - Prepare a PUF as described above without the exposure time during the sampling.

Air - analysis

- Perform a Soxhlet extraction with methanol (12 h);
- Concentrate the extract to 1 mL by using either a rotary evaporator or Kuderna-Danish;
- Filter the extract through a 0.2 µm glass hydrophilic polypropylene (GHP) filter into a polypropylene LC vial;
- Concentrate to 200 µL under a gentle stream of nitrogen;
- Add 100 µL injection standard 1;
- Add 300 µL 2mM ammonium acetate and shake manually;
- Analyze with LC-MS/MS .



Water sampling and sample preparation

The water sampling is described in “PFOS analysis in water for the Global Monitoring Plan of the Stockholm Convention” (UNEP GMP WG).

Sample preparation

- As soon as the sample arrives to the analytical laboratory internal standards (IS) should be added to compensate for absorbance to laboratory equipment;
- The sample (incl. IS) should have time to equilibrate before analysis;
- Keep the water samples (500 mL) in a high density polyethylene (HDPE) in the fridge or freezer (-20 °C) and defrost them the day before analysis;
- Shake the water rigorously before subsamples are taken out;
- Weigh 100 mL of water sample in a HDPE bottle (100 mL);

Procedural blank

- Prepare a procedural blank sample but using ultra clean (MilliQ) water as sample substitute

Human milk sampling and sample prep.

Follow the UNEP/WHO protocol for sampling of human milk 'UNEP-coordinated Survey of Mothers' Milk for Persistent Organic Pollutants' (http://www.unep.org/chemicalsandwaste/portals/9/POPs/docs/Mothers%20milk%20guide%20POPs.pdf)

Sample preparation

- Homogenise the samples (50 mL) manually by shaking for 1 min;
- Weigh 1 mL of milk sample, or 0.5 mL serum sample in PP tube (15 mL);
- Add 50 µL I.S. (4.2);
- Add 2 mL 50% formic acid and shake manually;
- Place the sample in an ultrasonic bath for 15 min;
- Centrifuge for 15 min at 3,000 rpm;
- Place the samples in an oven at 37 °C for 30 min.

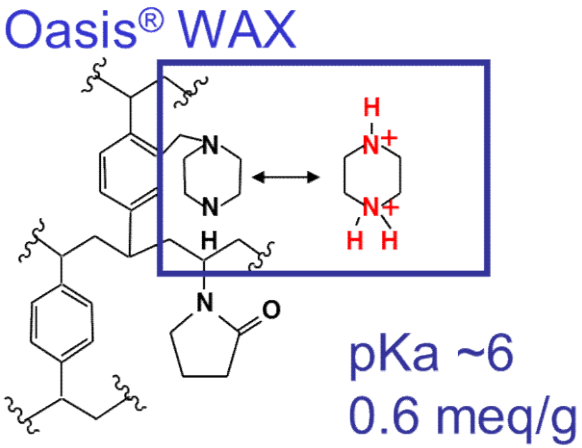
Procedural blank

- Prepare a procedural blank sample as described above in sample description but using ultra clean (MilliQ) water as sample substitute.

Water, human milk and human serum

- Solid phase extraction (SPE) is used for the extraction of water, mothers' milk and serum. Install an SPE cartridge on the SPE device.

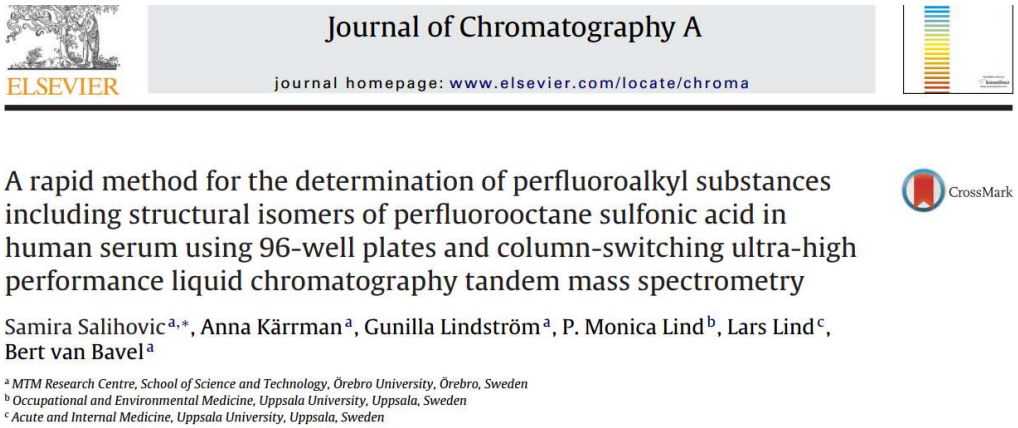
- Waters Oasis® WAX SPE Column
- Mixed-mode **W**eak **A**nion-e**X**change and reversed-phase sorbent
- Single use Oasis cartridge
- Retain and release strong acids (e.g. sulfonates)



Method validation and performance

- Sensitivity
  - MDLs ranged between 0.01 ng mL^{-1}-0.17 ng mL^{-1}
  - Linear range 0.01 ng mL^{-1}-60 ng mL^{-1}
- Accuracy
  - Conformed well with NIST SRM 1957 (n=54)
  - 6%-32% normalized difference

- Repeatability (QC n=7) and reproducibility (QC n=103) ranged between 2%-20% including structural PFOS isomers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Journal of Chromatography A
journal homepage: www.elsevier.com/locate/chroma

A rapid method for the determination of perfluoroalkyl substances including structural isomers of perfluorooctane sulfonic acid in human serum using 96-well plates and column-switching ultra-high performance liquid chromatography tandem mass spectrometry

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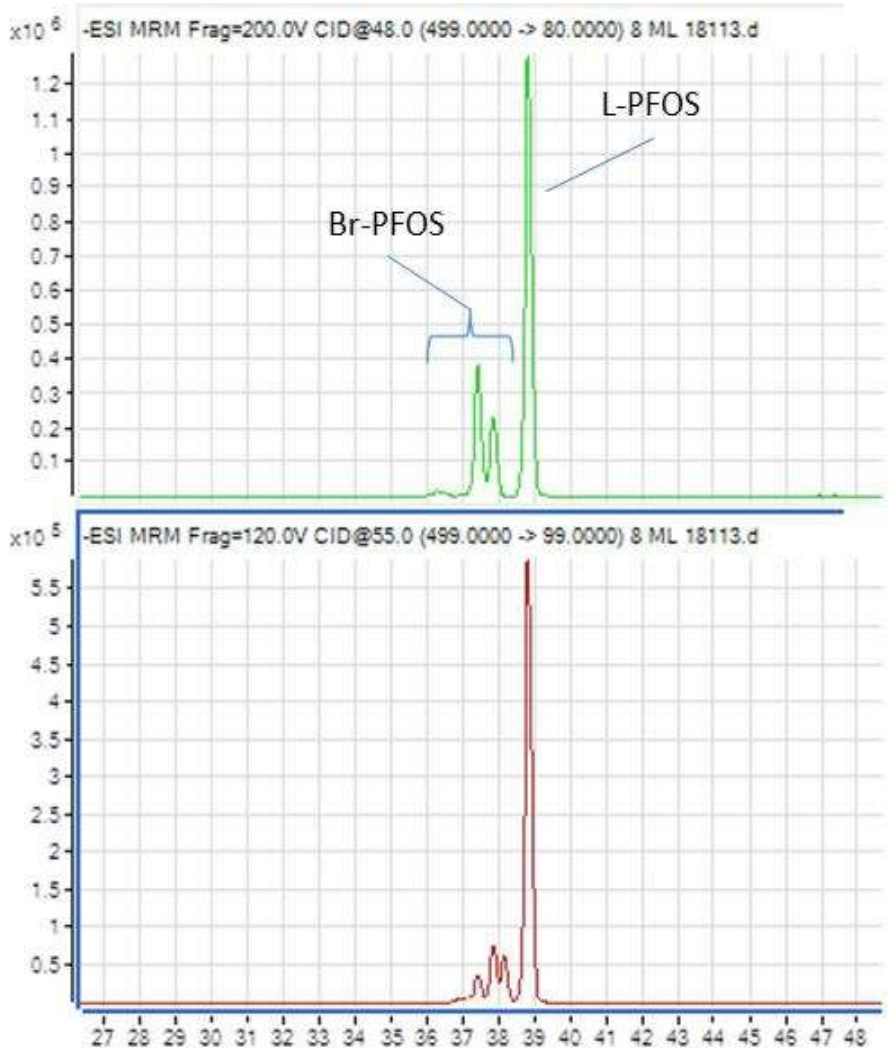
[b] Occupational and Environmental Medicine, Uppsala University, Uppsala, Sweden
[c] Acute and Internal Medicine, Uppsala University, Uppsala, Sweden

Instrumental analysis

- Install the analytical column and the guard column in the HPLC;
- Install an extra column (50 mm) and a guard column between the LC pump and the injector, to prevent interference of PFASs, originating from the LC system, with the target compounds;
- Purge all the mobile phase solvents through the system
- Start the pump with 65% ammonium formate and 35% methanol;
- Put all extracts, blanks, and calibration solutions in the tray of the autosampler;
- Make a sequence in the computer. Analyse the samples, the calibration solutions, the blank and the reference material in random order;
- Inject a calibration solution after pump has been running for at least 30 min;
- Check the performance of the LC-MS/MS by comparing the results (retention times and peak intensities) of the injected calibration solution with earlier results;
- Start the sequence.

Sample chromatogram



HF, New POPs Tools, Hanoi, Jan 2016

Chromatogram showing the separation of linear and branched PFOS in water (surface water sample from The Netherlands)

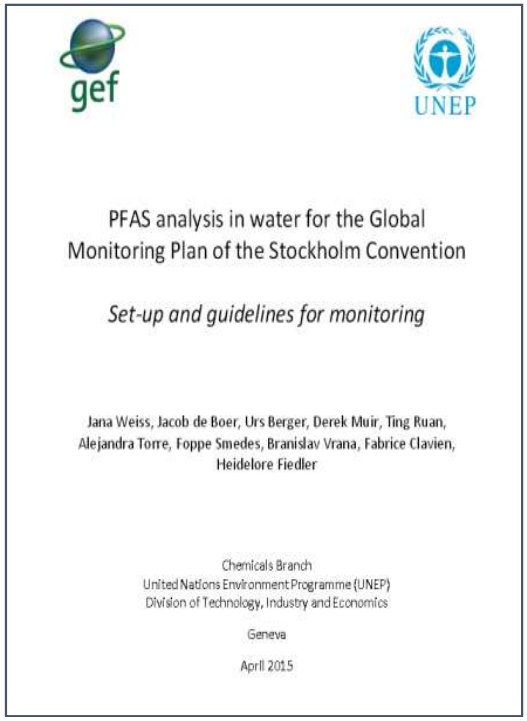
Note: PFAS concentrations should be reported on wet weight basis. However, often, results are reported on sulfonate anion basis, i.e., corrected for the molecular weight of the PFOS salt (with cation)

Mass settings for PFAS analysis

Compound		Precursor Ion (m/z)	Production (m/z)	Comment
PFOS	Target compound	499	80	Quantifier
			99	Qualifier
$^{13}C_4$ PFOS	Internal standard	503	80	Quantifier
			99	Qualifier
$^{13}C_8$ PFOS	Injection standard	507	80	Quantifier
			99	Qualifier
FOSA	Target compound	498	78	Quantifier
			169	Qualifier
$^{18}O_2$ FOSA	Internal standard	502	82	Quantifier
			169	Qualifier
MeFOSA	Target compound	512	169	Quantifier
			219	Qualifier
$^{2}H_3$ MeFOSA	Internal standard	515	169	Quantifier
			219	Qualifier
EtFOSA	Target compound	526	169	Quantifier
$^{2}H_5$ EtFOSA	Internal standard	531	169	Quantifier
MeFOSE	Target compound	602	45	Quantifier
$^{2}H_7$ MeFOSE	Internal standard	609	45	Quantifier
EtFOSE	Target compound	616	45	Quantifier
$^{2}H_9$ EtFOSE	Internal standard	625	45	Quantifier

Tools and methods for new POPs

- PFAS analysis in water - Set-up and guidelines for monitoring
- Instructive movie for analysis of PFOS and precursors
- Movie with instructions for the cleaning of PUF disks for passive sampling of ambient air



http://www.unep.org/chemicalsandwaste/POPsandScience/AnalysisandMonitoring/MethodDevelopment/tabid/1059865/Default.aspx